New Glycosyl Hydrolases from *Acidothermus cellulolyticus*

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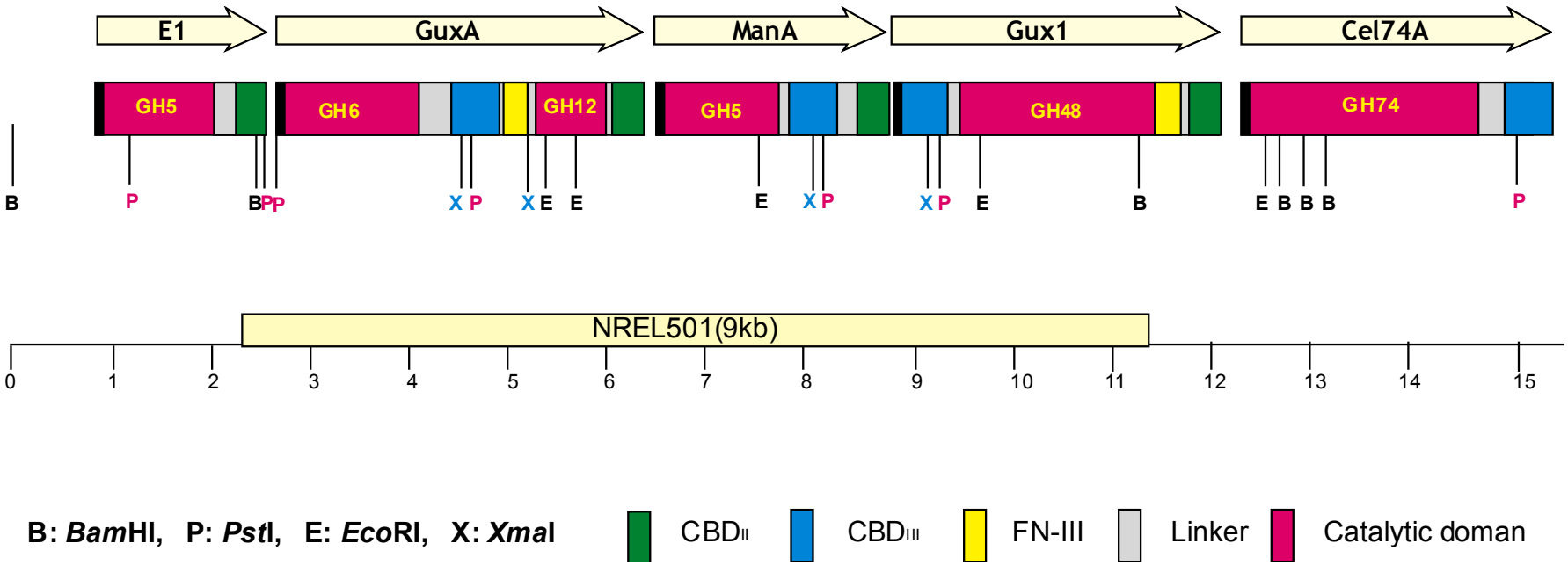
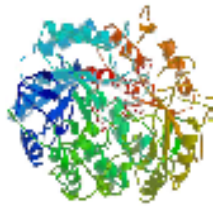
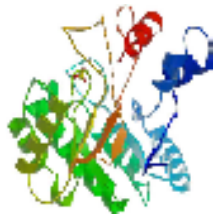
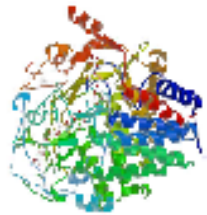
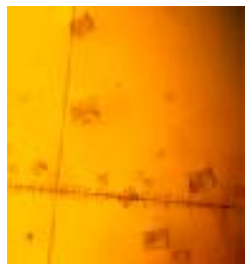
Abstract

Acidothermus cellulolyticus was isolated from 55-60^{o}C acidic water and mud samples collected in Yellowstone National Park in the early 1980s (Mohagheghi et al. *Int. J. System. Bacteriol.* 1986). Biochemical studies have shown that its hydrolytic enzymes are thermotolerant with maximal activities at temperatures of 75-83^{o}C. One of them, endoglucanase Cel5A, has been cloned and expressed in *E. coli* and other hosts. A lambda clone isolated from a genomic library of *A. cellulolyticus* grown on biomass was selected for DNA sequencing. A 9-kb *BamH*I fragment from this clone was subcloned into pDR540 and sequenced by primer walking. An inverse PCR technique was then applied to continue the sequencing of the genomic DNA and the primer walking method was used to sequence the large PCR products. Sequence analysis has revealed four additional ORFs downstream of the Cel5A gene. These genes all indicate glycosyl hydrolases with multidomain structure. Domains from GH families 5, 6, 12, 48, and 74 have been identified through sequence homology. Three of these enzymes have been cloned and expressed in *E. coli*. Some characteristics of the purified catalytic domains of the Cel 5a, Cel 12a and Cel 74 a are presented here.

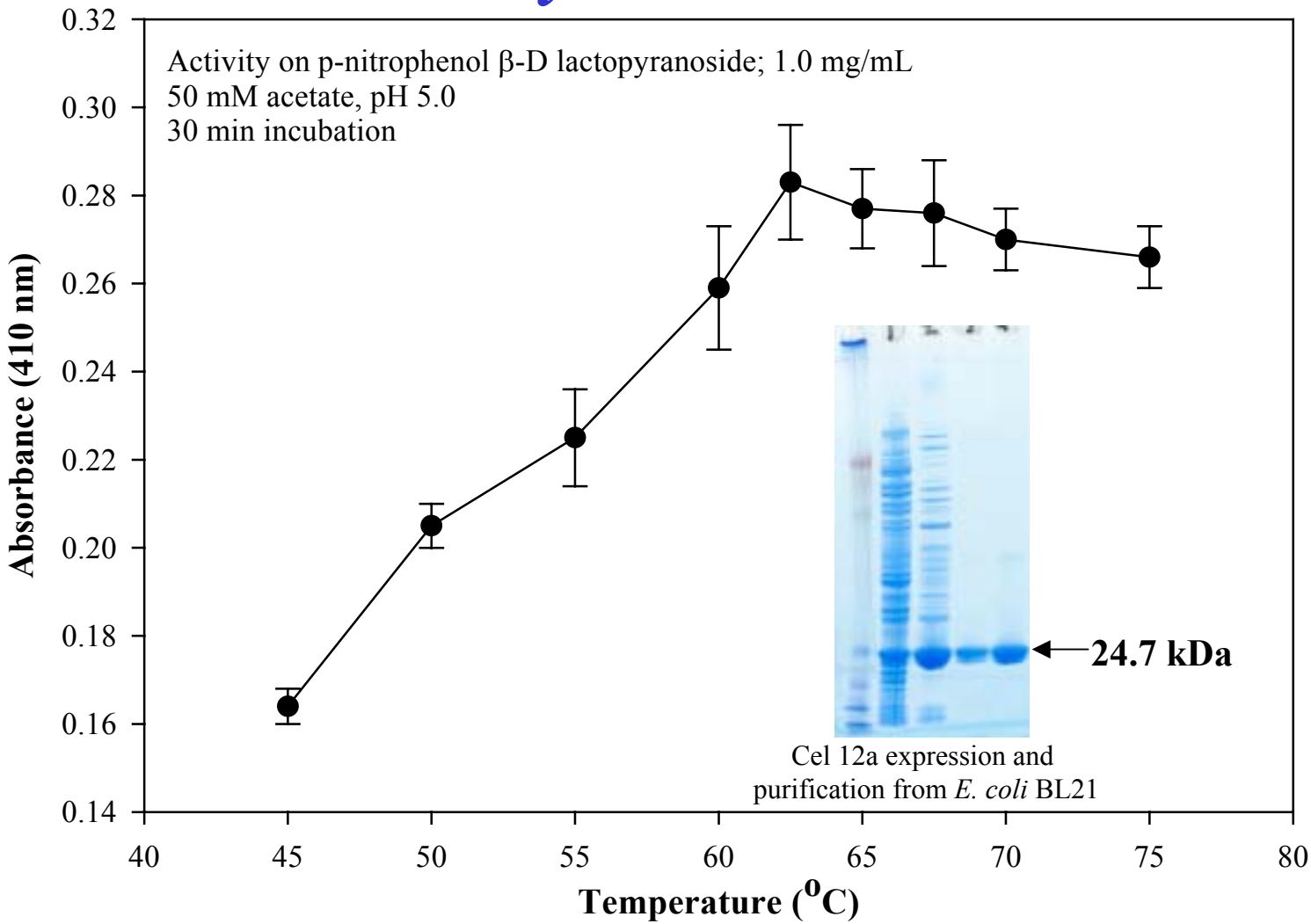
A Large Gene Cluster From the *A. cellulolyticus* Genome



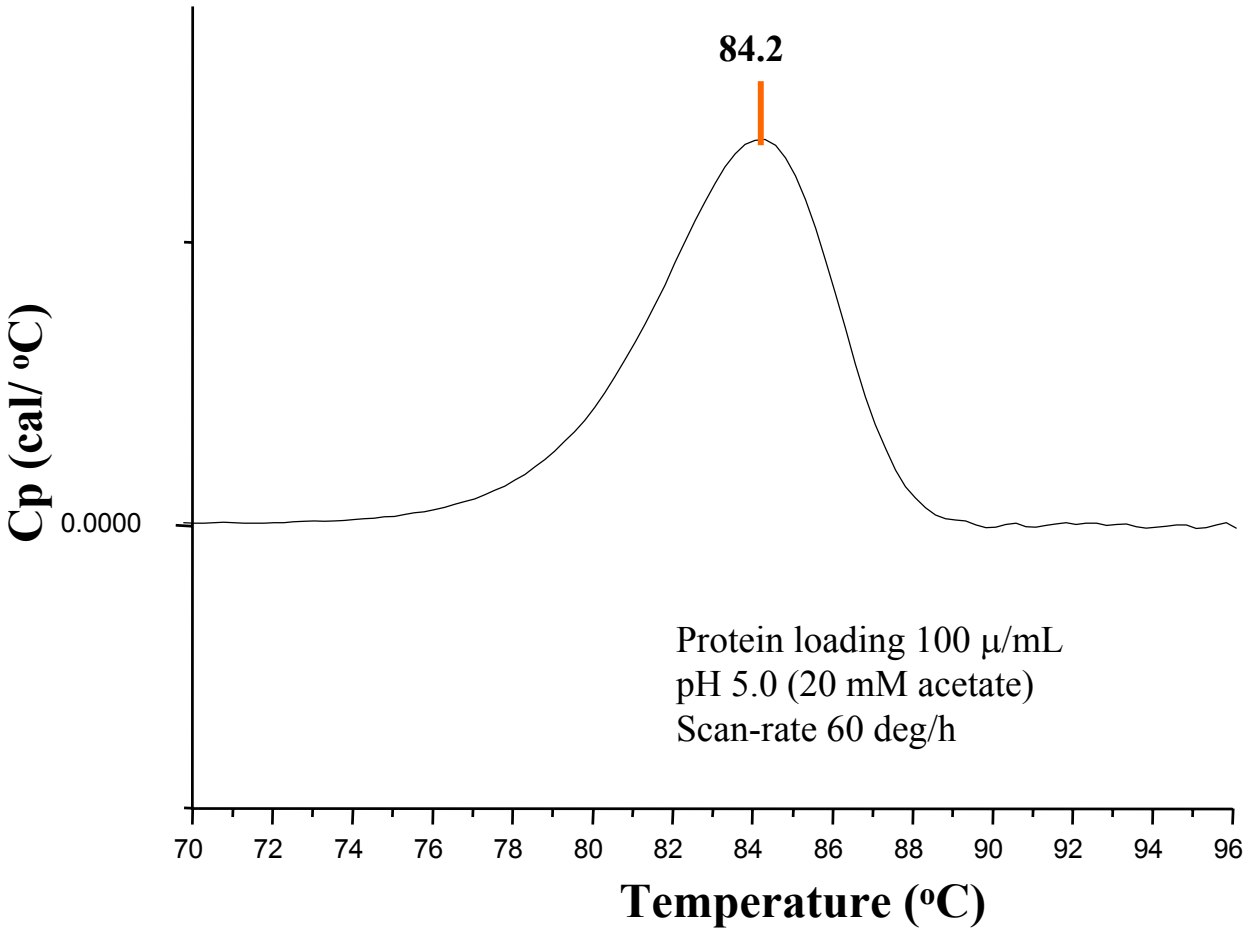
Related Glycosyl Hydrolases

Glycoside Hydrolase Families	5(Retention)	6(Inversion)	12(Retention)	48(Inversion)	74(Retention)
Structure example					
	Acidothermus cellulolyticus endoglucanase E1cd $(\beta/\alpha)_8$	*Thermobifida fusca* endoglucanase E2cd	*Streptomyces lividans* endoglucanase β-jelly roll	*Clostridium celluldyticum* celF $(\alpha/\alpha)_6$-helix barrel	
Aspergillus niger	endo gluca nase GH5		endogl ucan ase GH12		
Trichoderma reesei	endoglucanase EGII GH5 mannanase GH5	cellobioh ydrolase CBHII GH6	endogl ucan ase EGII GH12		
Clostridium thermocellum	endoglucanase CelE GH5 cell obiohydr olase CelO GH5 endog lucan ase CelC GH5 endog lucan ase CelB GH5 endog lucan ase CelG GH5 endog lucan ase CelG GH26 GH5			endoglucanase CelS GH48	
Thermobifida fusca	mann anase GH5 endogl ucanase E5 GH5	endogl ucan ase E2 GH6 cellobioh ydrolase E3 GH6		exoglucanase E6 GH48	cellulase GH74
Cellulomonas fimi	endog lucanase Cel5A GH5	cellobioh ydrolase CBHA GH6 endog lucanase CenA GH6		exocellobi ohydrol ase GuxB GH48	
Acidothermus cellulolyticus	endoglucanase E1 GH5 mannanase ManA GH5	cellobio hydrolase GuxA GH6 GH12	cellulase GuxA GH6 GH12	cellulase Gux1 GH48	cellulase AviII GH74

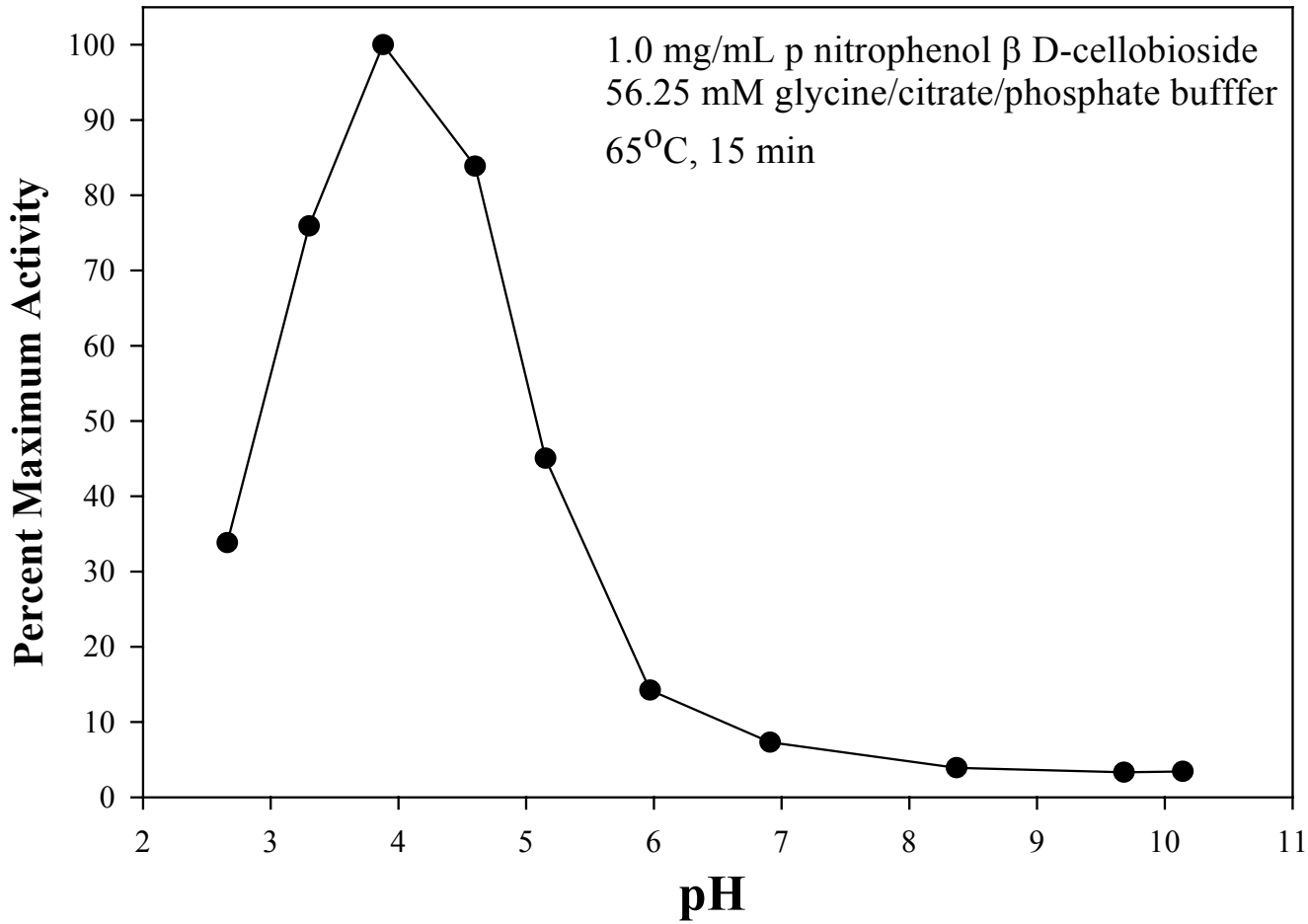
Temperature Optimum of *A. cellulolyticus* Cel 12A cd



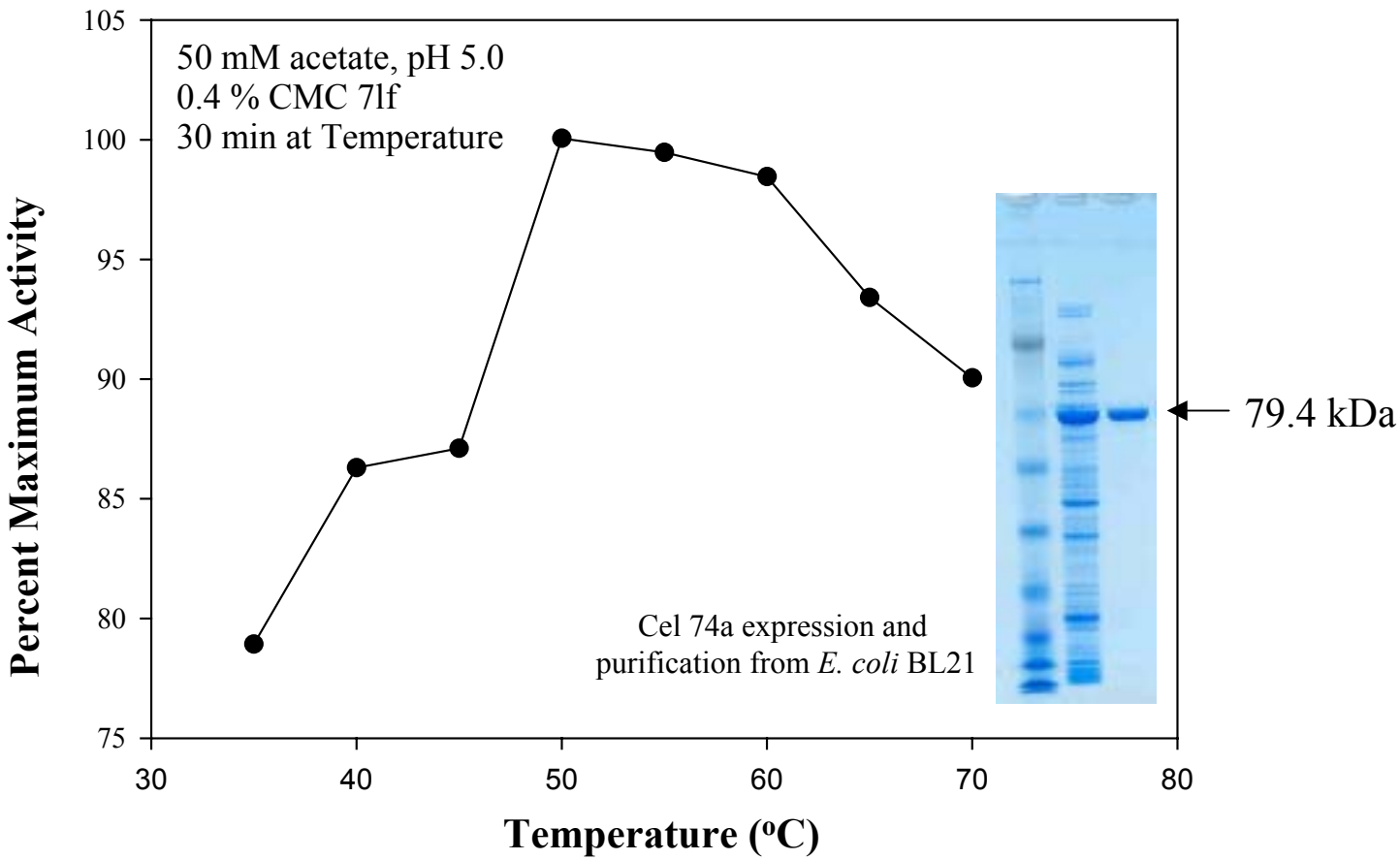
Differential Scanning microCalorimetry of *A. cellulolyticus* Cel 12A cd



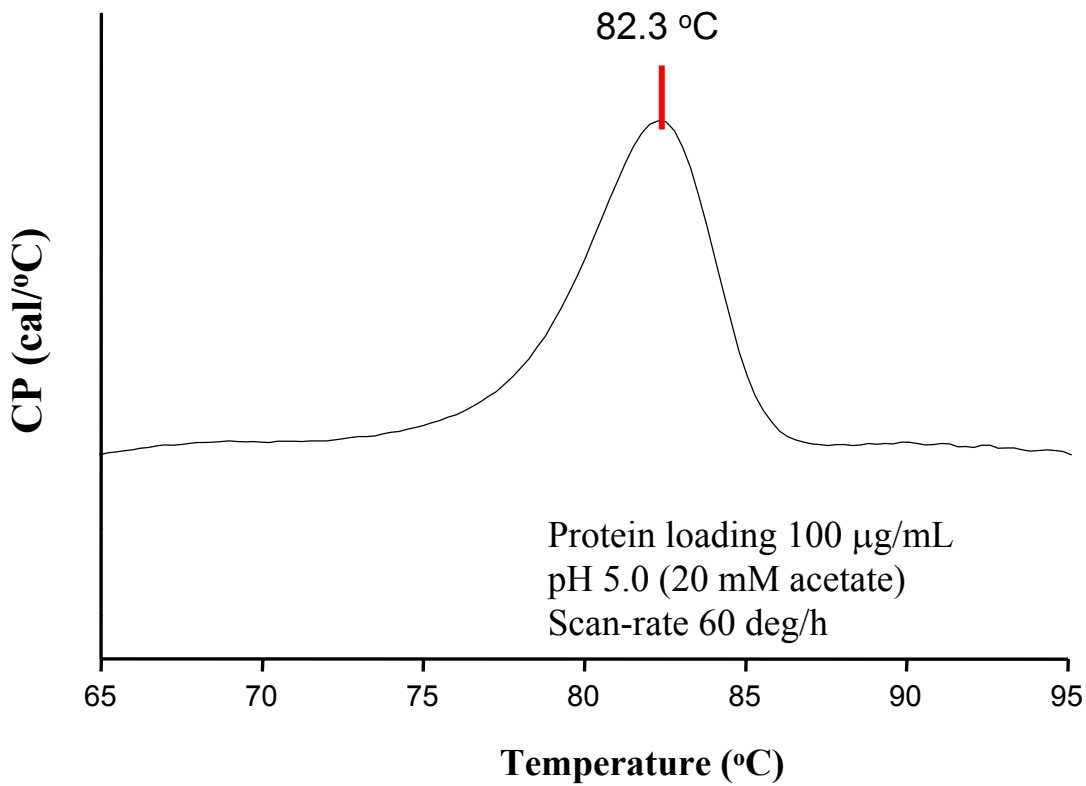
pH Optimum of *A. cellulolyticus* Cel 12A cd



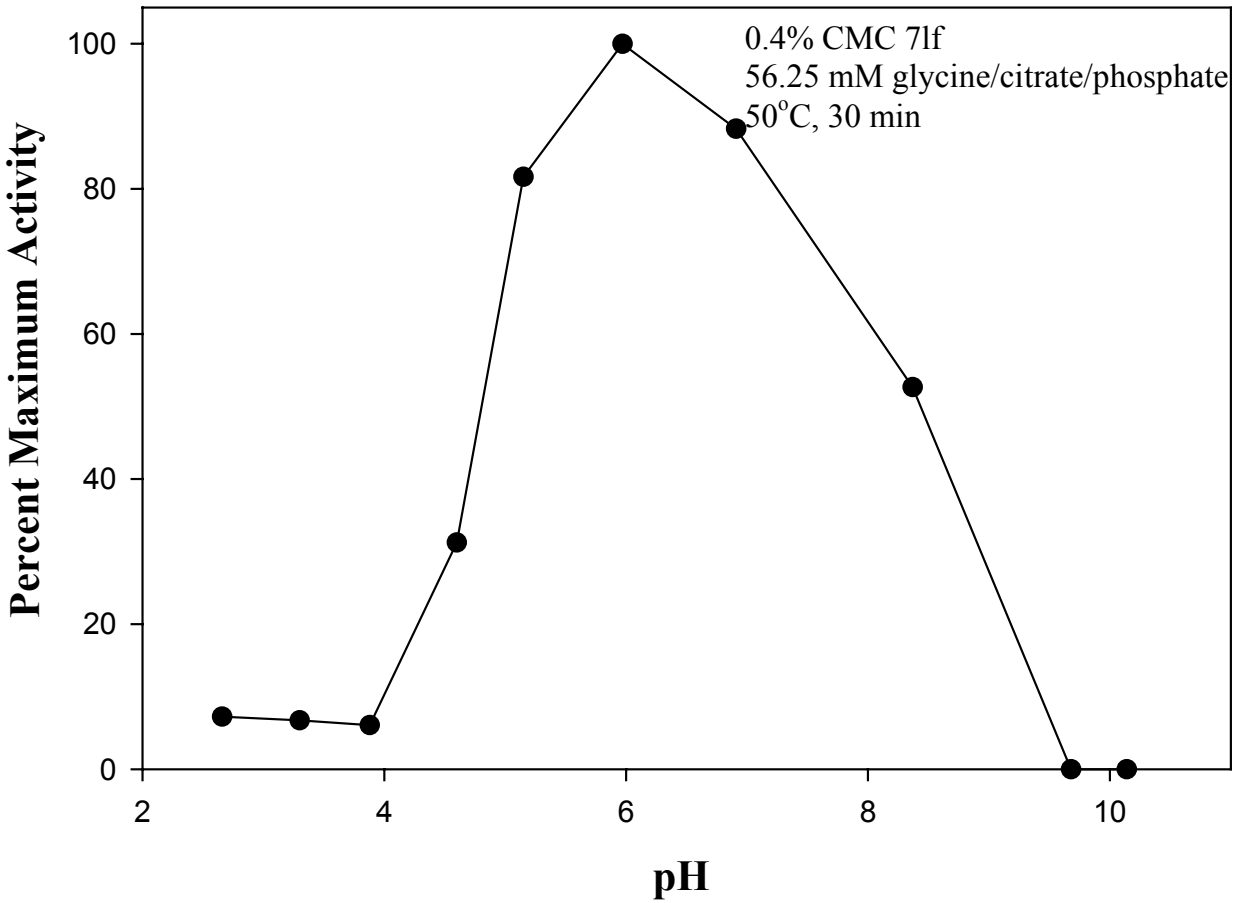
Temperature Optimum of *A. cellulolyticus* Cel 74A cd



Differential Scanning microCalorimetry of *A. cellulolyticus* Cel 74A cd



pH Optimum of *A. cellulolyticus* Cel 74A Catalytic Domain



Activity Comparison of *A. cellulolyticus* Glycosyl Hydrolases on Azurine-Crosslinked Insoluble Polysaccharides

AZCL-Substrate	Activity	cel5A 65°C	cel12A 65°C	cel74A 60°C
HE-Cellulose	*endo*-1,4-β-glucanase	4+	4+	4+
Galactomannan	*endo*-1,4-β-Mannanase	1+	-	-
Galactan	*endo*-1,4-β-Galactanase	-	-	-
β-Glucan	β-Glucanase	4+	4+	1+
Curdlan	*endo*-1,3-β-Glucanase	-	-	-
Arabinoxylan	*endo*-Xylanase	1+	2+	-
Xylan	*endo*-Xylanase	1+	3+	1+
Pachyman	*endo*-1,3-β-Glucanase	-	-	-
Xyloglucan	*endo*-1,4-β-glucanase	-	2+	4+
Dextran	*endo*-1,6-α-Dextranase	-	-	-
Amylose	α-amylase	-	-	-
Pullulan	Limit dextran	-	-	-

Activity = relative amount of dye released for each enzyme following a 1 hour incubation at temperature
Table is meant to demonstrate the substrate range for each enzyme.

Specific Activity (μmol/min mg^{-1}) of *A. cellulolyticus* Catalytic Domain Enzymes on p-nitrophenol Linked Soluble Substrates

PNP linked substrate	cel5A	cel12A	cel74A
β-D glucopyranoside	ND	ND	ND
β-D cellobioside	2.61	20.59	ND
β-D maltoside	ND	ND	ND
β-D xylopyranoside	ND	ND	ND
β-D mannopyranoside	ND	ND	ND
β-D lactopyranoside	0.117	6.63	ND

ND = not detectable
50 mM acetate, pH 5.0, 1.0 mg/mL substrate
60°C, 30 min

Conclusions

- Cellulase genes (*cel*12a and *cel*74a) from *Acidothermus cellulolyticus* were cloned by expression screening of a lambda genomic DNA library in *E. coli* using CMC.
- The catalytic domains from *cel* 12a and *cel*74a have been sequenced and expressed in *E. coli*. FASTA analysis of the catalytic domains classified the enzymes as a family 12 endoglucanase and a family 74 avicelase.
- The purified proteins are thermotolerant and their activities on soluble and insoluble dye-linked substrates were compared.

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